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Detection and Monitoring of Breast Cancer during
Therapy

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13. ABSTRACT (Maximum 200 Words) Purpose: Purpose was to develop non-invasive imaging systems to detect and treatment monitoring of breast cancer at the earliest stage in animal model. Scope: Scope of the second year's research is to perform in vitro and in vivo experiments, development of stable cell lines producing light-based reporters, development of additional viral vectors encoding dual reporter system, such as light based reporter (GFP, luciferase) and gamma camera based reporter (hSSTR2). Continue <i>in vivo</i> imaging in animal models with vector administration. Major findings: (1) Gamma camera imaging validated the newly developed light-based imaging. (2) Non-palpable breast tumor cells implanted in nude mice were detected non-invasively by light-based (bioluminescence) imaging. (3) Developed a novel technology to produce breast cancer cell lines that stably express GFP or luciferase. (4) Effects of adriamycin on breast cancer xenografts were detected non-invasively. (5) New vectors (Ad-GFP-hSSTR2-TK) were developed for further study on <i>in vivo</i> imaging. (6) Complement was found to play a major role in the delivery of vector to host and vector-host relation. Results: Normal looking non-palpable early stage breast cancer cells were detected and identified in animal models by light-based imaging. Results are presented and submitted for publication (please see the attachment). Significance: The present studies are highly significant for pre-clinical investigation on the early detection and treatment monitoring of breast cancer xenografts. This technology could be useful to assess the efficacy of new drugs against breast cancer in animal models. Complement plays a major role in response to viral vector administration into the host.				
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Introduction:

The ultimate goal of the present proposal is to apply novel technology of imaging to detect early stage breast cancer and monitor effects of anti-cancer agents against breast cancer in a non-invasive way in animal model. To accomplish our goals, a combination of multi-modality light-based imaging and gamma camera imaging was proposed. These imaging methods are complementary to each other. Light-based imaging provides high sensitivity and spatial resolution, including the potential for visualization of individual cells and subcellular structures. Light-based imaging technology is complementary to gamma camera imaging by providing molecular imaging of specific receptors and therapies in breast cancer.

Hypothesis: The hypothesis of the proposal was that a combined approach of light-based and gamma camera imaging will improve non-invasive early detection and monitoring of breast cancer.

The following Specific Aims are proposed to test the hypothesis:

1. Conduct *in vitro* studies to optimize strategies for imaging breast cancer using combined light-based imaging and gamma camera imaging.
2. Validate dual-modality imaging in animal models of breast cancer.
3. Apply dual-modality imaging to monitor therapeutic response in animal models of breast cancer.

In the first year, we reported that we successfully accomplished specific aim #1. In addition, we reported that parts of specific aims 2 and 3 were accomplished as well. In the present report we describe the accomplishments of tasks for second year and part of third year. In the following section the accomplishments are described.

Body: For second year parts of Task 2 and 3 were proposed. These tasks deal with the validation of dual-modality imaging in animal models of breast cancer and Apply dual-modality imaging to monitor therapeutic response in animal models of breast cancer. Following areas are covered.

Statement of Work proposed and approved by DOD.

Tasks 1. Conduct *in vitro* studies to optimize strategies for imaging breast cancer using combined light-based imaging and gamma camera imaging

- a. Development of new vectors. These are RGD-Ad-GFP (and RFP)-hSSTR2-TK, RGD-Ad-GFP (and RFP)-hSSTR2-CD, Ad-GFP-hSSTR2-TK, Ad-GFP-hSSTR2-CD and retroviral vector encoding GFP and stable transfectants with red fluorescence protein (RFP) or luciferase- (1st, 2nd and 3rd year).

Accomplishment: In the past year we have developed additional adenoviral vectors, Ad-GFP-hSSTR2-TK. These vectors are encoded for GFP and hSSTR2-TK.

Stable GFP and luciferase-positive breast cancer cell lines were developed. Human breast cancer cell line 2LMP, A subclone of MB-MDA231 was used to develop stably GFP and luciferase-positive cells. Luciferase is more sensitive than GFP. We developed a novel method to produce stably GFP or luciferase-positive breast cancer cells.

Task 2. Validate dual-modality imaging in animal models of breast cancer.

- a. Implant human breast tumor cells (variable cell numbers - range between 100 cells and 1 million cells) transfected with Ad-GFP-hSSTR2 and newly developed vectors from Task #1 in nude mouse subcutaneously and mammary fat pad to determine how early and what minimum cell numbers can be visualized in live mice. Fluorescence stereomicroscopy will determine the high resolution of GFP and gamma camera imaging will provide the high quantitative analysis of Tc-99m-labeled-P2045 bound to hSSTR2 on tumor cells.(2nd and 3rd year).

Accomplishment:

It was found that light-based imaging (stereomicroscopic and bioluminescence) was more sensitive and had higher resolution than gamma camera imaging. However, for imaging deeper tissue, nuclear imaging using Tc-99m-labeled-P2045 was necessary. A full range of imaging was performed using luciferase-positive human breast cancer cells. For in vivo imaging, a variable number of cells were implanted subcutaneously and in the mammary fat pad. Luciferase imaging was more sensitive than gamma camera imaging for detection of a minimum number of breast cancer cells that were implanted subcutaneously and in the mammary fat pad.

- b. Implant subcutaneously and in mammary fat pad breast tumor cells first in athymic nude mice followed by transfection with adenoviral vectors. Perform dual modality imaging. (2nd and 3rd year).

Accomplishment: Effective administration of for in vivo transfection of breast cancer cells by adenoviral vector is a very critical area in the gene therapy studies. Intravenous administration of Ad vector faces several obstacles. Complement is one of the major components. The effect of complement on transgene expression was evaluated *in vivo* and *in vitro* using mice lacking complement components. Complement component 3 (C3) deficient mice (C3^{-/-}) and appropriate wild type controls were intravenously injected with a replication incompetent, luciferase-expressing normal Ad5 (Ad5Luc1), or fibrin-fiber Ad5 (Ad5FFLuc1). Repeated, non-invasive bioluminescence imaging was conducted over 35 days. Our data shows for the first time that C3 facilitates both short- and long-term hepatic expression of luciferase following systemic delivery. C3^{-/-} mice showed significantly less ($p < 0.05$) luciferase expression in their liver than treatment-matched wild type mice when 2.3×10^9 (Ad5Luc1) and 4.0×10^9 (Ad5Luc1 or Ad5FFLuc1) viral particles (v.p.) were infused. The maximal difference in luciferase activity between C3^{-/-} and wild type mice was 99-fold difference at 3 days for the 2.3×10^9 v.p. dose (Ad5Luc1), 35-fold at 13 d for the 4.0×10^9 v.p. dose (Ad5Luc1), and 22-fold at 13 days

for the 4.0×10^9 v.p. dose (Ad5FFLuc1). Preincubation of Ad5Luc1 with wild type, C1q^{-/-}, or factor B (FB) deficient mouse sera for 5 min significantly ($p < 0.05$) increased transduction of mouse liver cells, as compared to preincubation with C3^{-/-} sera or PBS. These results suggest the classical or alternate complement pathway enhances Ad5 mediated liver transduction. These are significant findings for gene therapy research.

- c. Image anti-angiogenesis targeting *in vivo*. Inject intravenously a tracer dosage of anti-angiogenesis antibody DC101, dually labeled with Cy5.5 and Tc-99m, into mice bearing GFP-hSSTR2 labeled breast tumor cells at variable numbers. Collect images with stereomicroscopy to measure GFP at one wavelength, Cy5.5 at near infrared, and radioactivity by gamma camera imaging. Repeat the process at different time interval using the same mice (2nd and 3rd year).

Accomplishment: Breast cancer cells (stably luciferase-positive) cells were subcutaneously implanted in athymic nude mice. Anti-angiogenesis antibody DC101 dually labeled with Cy5.5 and Tc-99m was intravenously injected using tail vein. In a preliminary experiment dual modality imaging showed near infrared was more sensitive than gamma camera imaging to detect a small number of cells on the surface. The studies are in progress.

Task 3: Apply dual-modality imaging to monitor therapeutic response in animal models of breast cancer.

- a. Perform *in vivo* dual modality imaging of chemotherapy. - Image live mice to visualize the therapeutic intervention of adriamycin, tamoxifen and carboplatin to GFP-hSSTR2 positive breast tumor cells. (2nd and 3rd year). The studies are in progress.

Accomplishment: GFP-positive and luciferase-positive breast cancer cells were implanted in the mammary fat pad of athymic female nude mice followed by therapeutic intervention with adriamycin and tamoxifen. Tumor cells/lesions were visualized and detected non-invasively long before any solid tumor was visible or palpable. Light-based imaging showed a great potential for studying the effects of drugs on breast cancer in animal model. The localization of anti-cancer drugs was imaged by fluorescent stereomicroscopy.

- b. Perform *in vivo* dual modality imaging of anti-angiogenesis therapy. - Image live mice (implanted with breast tumor cells transfected with Ad-GFP-hSSTR2 before and after implantation) to visualize the effects of dual-labeled (Cy5.5-Tc-99m) anti-angiogenesis antibody DC101 on GFP-positive breast tumor cells (2nd and 3rd year).

Accomplishment: GFP-positive breast cancer cells were implanted in the mammary fat pad of athymic nude mice followed by dual-labeled anti-angiogenesis antibody DC102. Breast tumors were detected using dual modality imaging system. At the same time GFP-positive cells were imaged to visualize the location of tumors in mice. The studies are in progress.

Key Research Accomplishment:

- Non-invasive light-based imaging methods were developed with stereomicroscope and bioluminescence. The work was presented in the annual meeting of American Association for Cancer Research in March, 2004 (Abstract published in - Appendix I).
- Light-based imaging was shown to be highly sensitive to detect disseminated breast cancer in animal model during therapeutic intervention. This work as an abstract was published in American Society of Clinical Oncology - Appendix II).
- Effective administration of for *in vivo* transfection of breast cancer cells by adenoviral vector is a very critical area in the gene therapy studies. Intravenous administration of Ad vector faces several obstacles. Complement is one of the major components. The effect of complement on transgene expression was evaluated *in vivo* and *in vitro* using mice lacking complement components. (Manuscript accepted in Gene Therapy, 2004).
- Gamma camera imaging validated the newly developed light-based imaging.
- Non-palpable breast tumor cells implanted in nude mice were detected non-invasively by light-based (bioluminescence) imaging.
- Developed a novel technology to produce breast cancer cell lines that stably express GFP or luciferase.
- Effects of adriamycin on breast cancer xenografts were detected non-invasively.
- New vectors (Ad-GFP-hSSTr2-TK) were developed for further study on *in vivo* imaging.

Reportable outcomes:

Manuscript (Accepted): Bioluminescence imaging reveals a significant role for complement in liver transduction following intravenous delivery of adenovirus. Gene Therapy, 2004.

Manuscript in preparation:

Dual modality imaging for the early detection and treatment monitoring of breast cancer in animal model.

A novel method to produce stably GFP-positive and luciferase-positive breast cancer cells.

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum and Kurt R. Zinn.

Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy.

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, and Kurt R. Zinn. Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging (BI).

Abstracts Presented:

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum and Kurt R. Zinn. Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy. AACR. March, 2004, in Orlando Florida.

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, and Kurt R. Zinn. Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging (BI). ASCO, June 2004, in New Orleans, Louisiana.

Chaudhuri TR, Viral delivery of light-based reporters for detection and monitoring of disease. Mol Imaging Biol 6(2): 69, 2004. (Invited).

Chaudhuri, TR, V. N. Krasnykh, Z. Cao, A. Stargel, P. L. Simhadri, N. Belousova, K. R. Zinn. Intravenous administration of adenoviral vector detected metastatic breast cancer by bioluminescent imaging in live mice. Journal of International Conference on Gene Therapy of Cancer, December 10-14, 2003, San Diego, California.

Abstracts Published:

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum and Kurt R. Zinn. Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy. Proceedings :AACR. March, 2004.

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, and Kurt R. Zinn. Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging (BI). Proceedings: ASCO, June 2004.

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Chaudhuri, TR, V. N. Krasnykh, Z. Cao, A. Stargel, P. L. Simhadri, N. Belousova, K. R. Zinn. Intravenous administration of adenoviral vector detected metastatic breast cancer by bioluminescent imaging in live mice. Journal of International Conference on Gene Therapy of Cancer, December 10-14, 2003, San Diego, California.

Development of cell lines: GFP-positive breast cancer cell line (2LMP-GFP). Luciferase-positive breast cancer cell line (2LMP-luc).

Funding applied for based on work supported by this grant: Partial results of the present research helped to apply for additional funding from Sankyo Co. Ltd. to study the efficacy of a newly developed drug against breast cancer. Proposal has been funded.

Personnel supported by this grant:

Dr. Zhihong Cao, Research Associate

Ms. Amanda Stargel, Research assistant
Ms Glorisa Reason, Laboratory technician.

Conclusions: The light-based imaging continues to show great potential in the detection and treatment monitoring of breast cancer in a non-invasive manner. GFP, RFP, Luciferase or Cy 5 has significant contributing factors in this research. The efficacy of fluorescent stereomicroscopy was validated by gamma camera imaging. Both light-based and gamma camera imaging can detect the breast cancer in animal. However, a contrast such as GFP, RFP is necessary to identify few cancer cells behind millions of normal cells. This is true in vitro as well as in vivo system. The present studies are highly significant for pre-clinical investigation on the early detection and treatment monitoring of breast cancer xenografts. This technology could be useful to assess the efficacy of new drugs against breast cancer in animal models. Complement plays a major role in response to viral vector administration into the host.

References: Publications and Abstracts:

Kurt R. Zinn, Alexander J. Szalai, Amanda Stargel, Victor Krasnykh, **Tandra R. Chaudhuri**. Bioluminescence imaging reveals a significant role for complement in liver transduction following intravenous delivery of adenovirus. Accepted. Gene Therapy, 2004.

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum and Kurt R. Zinn. Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy. Proceedings :AACR. March, 2004.

Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, and Kurt R. Zinn. Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging (BI). Proceedings: ASCO, June 2004.

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Appendices:


Appendix I: Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum and Kurt R. Zinn. Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy. Proceedings: AACR. March, 2004.

Appendix II: Chaudhuri TR, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, and Kurt R. Zinn. Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging (BI). Proceedings: ASCO, June 2004.

Appendix III: Kurt R. Zinn, Alexander J. Szalai, Amanda Stargel, Victor Krasnykh, **Tandra R. Chaudhuri**. Bioluminescence imaging reveals a significant role for complement in liver transduction following intravenous delivery of adenovirus. Accepted. Gene Therapy, 2004.

Appendix IV:

CV – Dr. Tandra Rani Chaudhuri.

 [Print this Page for Your Records](#)[Close Window](#)**Control/Tracking Number :** 04-AB-6664-AACR**Activity :** Abstract Submission**Current Date/Time :** 11/7/2003 10:37:37 PM**Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy**

Tandra R. Chaudhuri, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, Kurt R. Zinn. University of Alabama at Birmingham, Birmingham, AL

Background

While TRA-8, an anti-DR5 antibody, induces apoptosis in TRAIL-sensitive tumor cells, no sensitive non-invasive methods are available to monitor the treatment effects on minimal disease in animal models. Bioluminescence imaging allows quantitative assessment of a small number of cells, and monitoring the tumor growth and treatment response by detecting the light emitted from tumor cells expressing the firefly luciferase (Luc) as a reporter gene. We report our non-invasive monitoring of multimodality treatments against non-palpable breast cancer xenografts in live animals.

Aim

The purpose of the study was to apply bioluminescence imaging to detect treatment effects in non-palpable breast tumors in mice. TRA-8 antibody and Adriamycin were tested alone, or in combination.

Methods

To accomplish our goals, the human breast tumor cell line 2LMP (a subclone of MDA-MB231 obtained from M. Lippman, Univ. of Michigan) was transfected with adeno-associated virus encoding firefly Luc gene, and a stable Luc-positive 2LMP (Luc-2LMP) cell line was established by screening clones in 96-well plates using a Xenogen IVIS-100 imaging system. In vitro studies established that the Luc-2LMP cell line responded identically to treatments, as compared to the parent cell line. For in vivo studies, four groups (4/group) of athymic female nude mice were implanted with Luc-2LMP cells (1 million/mouse) in the mammary fat pad. Treatments included combined TRA-8 (100-200 microgram/mouse)/Adriamycin (6 mg/kg) (Gr 1), TRA-8 (Gr 2), Adriamycin (Gr 3), and untreated controls (Gr 4). All treatments were given intravenously (2X/week for 4 wks). Mice were imaged over time with the IVIS-100, and tumor mass was determined by measuring light transmitted from the Luc-positive tumors.

Results

Bioluminescence imaging demonstrated high sensitivity for non-invasive detection and treatment monitoring of non-palpable breast tumors in mice. The measurement of light emission from stable Luc-2LMP cells in mice allowed tracking the tumor regression during therapy. Real time imaging data revealed significant inhibition of tumor growth in all treatment groups relative to controls, while the combined treatment with TRA-8/Adriamycin was most effective. ~ 95% of tumor cells were killed after the first two doses of combination therapy.

Conclusions

Bioluminescence imaging was applied to evaluate a combined TRA-8/Adriamycin strategy for apoptosis induction in breast cancer xenografts. This method can be more widely applied for new drugs and combinations for cancer therapy, enabling non-invasive assessment of tumor cell killing over time. (Supported in part by Sankyo Co., Ltd, DAMD17-02-1-0264, and DAMD17-02-1-0266, NIH grant # CA80104.)

Author Disclosure Block: T.R. Chaudhuri, None; Z. Cao, None; S. Ponnazhagan, None; A. Stargel, None; P.L. Simhadri, None; T. Zhou, None; A.F. LoBuglio, None; D.J. Buchsbaum, None; K.R. Zinn, None.

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Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging

T. R. Chaudhuri, Z. Cao, S. Ponnazhagan, A. Stargel, P. L. Simhadri, T. Zhou, A. F. LoBuglio, D. J. Buchsbaum, K. R. Zinn; University of Alabama at Birmingham, Birmingham, AL

Background: TRA-8, a mouse anti-human DR5 monoclonal antibody induces apoptosis in TRAIL-sensitive tumor cells. However no non-invasive method is available to monitor the treatment effects on disseminated cancer. We report a non-invasive and sensitive BI technology to determine the efficacy of combined treatment of TRA-8 and Adriamycin on disseminated breast cancer in animal model. **Methods:** Human breast cancer cell line 2LMP, a subclone of MDA-MB-231, was transfected with adeno associated virus encoding luciferase (AAV-luc). A stable luciferase-positive cell line was established by screening. Two groups (5/group) of athymic female nude mice were used. Four sites (liver, spleen, chest cavity and peritoneum) of each mouse were injected with luciferase-positive 2LMP cells (0.25×10^6 /site). After 7 days, BI revealed disseminated tumor sites; one group of mice was injected i.v. with 150 μ g TRA-8 and 6 mg/kg Adriamycin, and the 2nd group of mice did not receive any treatment. All treatments were given 2X/wk for 3 wks. Mice were imaged over time with an IVIS-100 Xenogen imaging system and tumor mass was estimated from ventral, dorsal, left and right lateral views by measuring light transmitted from the luciferase positive tumors. **Results:** All untreated mice died by day 21 with 4-6 fold increases in bioluminescence and extensive metastasis, including bone. Treated mice had a dramatic decrease in bioluminescence to 15-20% of day 7 values that persisted for 45 days and then began a progressive regrowth of tumor (increasing bioluminescence) in multiple sites. Dissemination of breast cancer was detected by BI and confirmed by dissection. In all untreated mice, numerous tumor nodules were detected in lungs, heart, pleural space, bones including ribs and spine, diaphragm, esophagus and pericardium, liver, spleen, stomach, kidneys, ovaries, uterus, and peritoneal membrane. **Conclusions:** BI demonstrated high sensitivity for non-invasive detection and treatment monitoring of disseminated breast tumors in mice. Combination therapy with TRA-8 and Adriamycin was highly effective in the regression of disseminated breast cancer in this animal model. (Supported by DAMD17-02-1-0266, NIH CA80104, DAMD17-02-1-0264, Sankyo CO,Ltd.)

**Bioluminescence imaging reveals a significant role for complement in liver transduction
following intravenous delivery of adenovirus**

REVISED #2

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Running Title: Complement and liver transduction by adenovirus

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complement

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Summary

The effect of complement on transgene expression was evaluated *in vivo* and *in vitro* using mice lacking complement components. Complement component 3 (C3) deficient mice (C3^{-/-}) and appropriate wildtype controls were intravenously injected with a replication incompetent, luciferase-expressing normal Ad5 (Ad5Luc1), or fibrin-fiber Ad5 (Ad5FFLuc1). Repeated, non-invasive bioluminescence imaging was conducted over 35 days. Our data shows for the first time that C3 facilitates both short- and long-term hepatic expression of luciferase following systemic delivery. C3^{-/-} mice showed significantly less ($p < 0.05$) luciferase expression in their liver than treatment-matched wildtype mice when 2.3×10^9 (Ad5Luc1) and 4.0×10^9 (Ad5Luc1 or Ad5FFLuc1) viral particles (v.p.) were infused. The maximal difference in luciferase activity between C3^{-/-} and wildtype mice was 99-fold difference at 3 days for the 2.3×10^9 v.p. dose (Ad5Luc1), 35-fold at 13 d for the 4.0×10^9 v.p. dose (Ad5Luc1), and 22-fold at 13 days for the 4.0×10^9 v.p. dose (Ad5FFLuc1). Preincubation of Ad5Luc1 with wild-type, C1q^{-/-}, or factor B (FB) deficient mouse sera for 5 min significantly ($p < 0.05$) increased transduction of mouse liver cells, as compared to preincubation with C3^{-/-} sera or PBS. These results suggest the classical or alternate complement pathway enhances Ad5 mediated liver transduction.

Activation of innate immunity and promotion of inflammation are common responses to replication incompetent adenoviruses (Ad) now being developed as vectors for gene therapy.^{1,2} The complement system is central to both innate immunity and inflammation.^{3,4} Because it is comprised of multiple membrane-bound and blood-borne factors, the complement system is probably of particular relevance in delivery of vectors administered intravenously. In fact, Cichon *et al* showed complement was activated in a majority of human plasma samples when challenged with different adenoviral serotypes; complement activation was completely dependent on anti-Ad antibody.⁵ Based on these studies, the suggestion was made that complement activation would not be a problem for local delivery of low Ad doses, but high doses of Ad administered by a systemic route could have potentially adverse consequences.⁵

Complement "activation" is a complex series of enzymatic reactions that converts pre-existing protein substrates into biologically active end-products. For example, in a process called opsonization, the deposition of C3 fragments onto pathogens promotes the removal of the pathogens by the reticuloendothelial system. In gene therapy applications, redirection of the vector in this manner might lead to toxicity. Equally important, less vector would remain available for transfecting the desired target cell population. Consistent with this view, Wilson *et al* reported greater reporter expression in mouse hepatocytes following systemic Ad administration with high vector doses that saturated Kupffer cells.⁶ This was true even with doses that included a different Ad without the reporter construct.⁶

It is widely accepted that the liver is the predominant site of reporter gene expression following intravenous injection of wild-type Ad5 vectors.⁷ Coxsackie and

adenovirus receptor (CAR), integrins, and heparin sulfate proteoglycans have all been shown to be important for liver transfection.⁷⁻¹² Ad vectors with CAR binding site mutations and ablation of integrin-binding showed less luciferase expression in liver following systemic administration.⁷ Similarly, ablation of CAR-binding via short fiber replacements also lead to reduced liver tropism.¹¹ More recently it was reported that blood coagulation factor IX was also involved in liver transduction.¹³ The humoral immune response also influences liver transgene expression, especially when the host is repeatedly exposed to the vector, because neutralizing antibody can diminish liver transfection.

The data reported herein show for the first time the importance of complement in the transduction of mouse liver by Ad. To directly address the role of complement in liver transduction, we performed studies using wildtype mice versus mutant mice unable to make complement component 3 (C3).¹⁴ By repeated bioluminescence imaging of living mice, we assessed liver luciferase expression following intravenous delivery of the Ad vector. Surprisingly, at low Ad5Luc1 doses, C3 deficient mice (C3^{-/-}) showed up to 99-fold less luciferase expression in the liver compared to wildtype controls, indicating a facilitatory role for the complement pathway in liver transduction. The current experiments used C3^{-/-} mice with the C57BL/6 background, together with littermate controls (homozygous C3^{+/+}) matched for sex and age (hereafter, wildtype or control).

In Figure 1 we present representative images captured from mice that received the lowest dose (2.3×10^9 v.p.) of Ad5Luc1. Each image (1-min acquisition) was collected on day 13 after Ad5Luc1 delivery; the pseudocolor overlay represents the intensity of light emission, and thus the level of luciferase expression. Overall, wildtype

mice showed 12.7-fold greater liver luciferase expression than $C3^{-/-}$ mice at this time point, and the absolute difference was statistically significant ($p < 0.05$, ANOVA).¹⁵

With all 3 doses of Ad5Luc1, peak liver luciferase expression in both kinds of mice was detected on day 6-10 (Fig. 2). Maximal luciferase expression ranged from 10- to 100-times greater than that observed 1-2 d after vector administration. Wildtype mice always showed higher liver luciferase expression, but the absolute difference between wildtype and $C3^{-/-}$ mice was diminished as the dose of Ad5Luc1 was increased. For example, liver luciferase expression 3 days after injection of 2.3×10^9 v.p. was 99-fold higher in wildtype mice compared to $C3^{-/-}$ mice (Fig. 2A). For mice injected with 4.0×10^9 v.p. (Fig. 2B), wildtype mice showed 35-fold higher liver luciferase expression compared to $C3^{-/-}$ mice. For the highest Ad5Luc1 dose (1.3×10^{10} v.p.), the maximal difference between the two groups was 3.4-fold (Fig. 2C). For the $C3^{-/-}$ mice in isolation, significantly greater luciferase expression in the liver was observed with increasing Ad5Luc1 vector dose. In contrast, the control mice with an intact complement system did not show greater luciferase expression with increasing Ad5Luc1 dose.

An experiment was performed in the two groups of mice using intravenous Ad5FFLuc1 (4.0×10^9 v.p.). Ad5FFLuc1 is a fiber-fibritin Ad5 encoding Luc1 and does not support CAR-dependent pathways of infection.¹⁶ As shown in Figure 3, the wildtype mice averaged higher levels of liver luciferase expression compared with $C3^{-/-}$ mice; maximal difference was 22-fold at 17 days after injection. These data suggest the complement effect on liver transfection is independent of CAR-mediated mechanisms.

Complement appeared to facilitate liver transduction as C3^{-/-} mice always showed lower luciferase expression than wildtype mice. The facilitation effect was overcome if high numbers of the vector were injected, thus C3^{-/-} and wildtype mice showed similar liver luciferase expression after administration of 1.3×10^{10} Ad5Luc1. Importantly, none of the mice used in the present study were previously exposed to Ad vectors, so the complement-dependent effect was independent of an antibody response against the Ad vector. Also, the complement-dependent effect did also not require CAR-dependent routes of infection, as demonstrated using Ad5FFLuc1.

Complement activation may lead to opsonization of the vector, binding of the complement-coated vector to cells, and subsequent infection. To test this hypothesis, *in vitro* studies were conducted with Ad5Luc1 and fresh serum from transgenic mice lacking complement components. Mouse liver hepatoma (TIB76) cells were incubated with the Ad5Luc1 following preincubation for 5 min with the various sera. As shown in Figure 4, preincubation of Ad5Luc1 with C3^{-/-} sera resulted in luciferase levels in the TIB76 cells that were not significantly different from levels in TIB76 cells treated with Ad5Luc1 preincubated with PBS. Importantly, preincubation of Ad5Luc1 with C3^{-/-} serum resulted in significantly less ($p < 0.05$) luciferase expression (3-fold) compared to preincubation with wildtype serum. Remarkably, preincubation with C1q^{-/-} and FB^{-/-} sera achieved intermediate levels of transduction in the TIB76 cells.

Our *in vitro* and *in vivo* findings suggest that C3, and likely its activation via either a FB- or C1q-dependant pathway, enhances Ad5 mediated transduction of the liver or liver cells. It appears that opsonization of the Ad5 particle itself facilitates transfection, which would point to a potential cellular complement receptor as another important

component of this mechanism. While the current work does not identify this receptor, it provides strong evidence for its existence. Of interest, it was recently reported that group B adenovirus uses CD46, a complement regulatory protein, for cellular attachment.¹⁷ Therefore, the current report provides another example of an adenovirus exploiting a complement pathway for infection. As such, a strong immune response may be a consequence. This is in contrast to other pathways by which pathogens exploit the complement system as a means to escape the host immune response.¹⁸⁻²³ For example, HIV has evolved to exploit complement pathways to survive and promote transmission to permissive cells.²⁴

In order for systemic delivery of Ad vectors to achieve clinical practicality, a better understanding of innate immunity is needed, including how complement influences the transduction process. Our findings suggest that inhibition of complement may be a valid approach to overcome the liver's propensity to remove systemically administered Ad, as well as an approach to reduce the strong immune response to the Ad vector. Two reports from another group are supportive of this concept, as complement depletion improved intravascular delivery of replication-conditional Herpes virus to human xenograft tumors growing in rat brain.^{25,26} While Ikeda *et al* utilized a different virus and model system, their findings of improved delivery of virus to the brain xenografts following complement inhibition is consistent with reduced opsonization and reduced liver sequestration. Less inactivation and removal of virus would make more available to target the xenograft brain tumors. In addition to benefits for tumor targeting, inhibition of complement activation may also have the added benefit of decreasing the humoral and cell mediated immune response to virus.²⁷ It is envisioned that future Ad vectors

will display complement regulatory proteins on their surface, or other surface proteins capable of binding negative regulators of complement activation in host blood. Potential sites of incorporation of these proteins in the Ad include the hexon, or pIX, a recently demonstrated site for genetic addition of peptides.²⁸ A linker site (poly GGGGS) between the FF chimera and retargeting ligands is another potential site.¹⁶ In this manner, negative regulators of complement activation would be present on the surfaces of the Ad vector. Complement activation would thereby be reduced, potentially minimizing undesired toxicities (inflammation, immune response) and/or improving targeting outcomes to tissues other than liver. In combination with other efforts to modify the vector for targeting, efforts to interfere with complement should be considered.

Acknowledgements.

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Figure Legends.

Figure 1: Bioluminescence imaging of luciferase expression in living mice at 13 days after intravenous injection of 2.3×10^9 v.p. of Ad5Luc1 in (A) wildtype control mice, and (B) C3^{-/-} mice. The pseudocolor overlay represents the intensity of light emission, and thus the level of luciferase expression. A single lot of E1-deleted recombinant Ad5Luc1 containing the firefly luciferase gene under control of CMV promoter was used;²⁹ all injections of the virus were intravenous. Preliminary studies with a range of

Ad5Luc1 doses (2×10^9 - 1×10^{10} v.p.) showed from 10- to 100-fold less expression of luciferase in $C3^{-/-}$ mice (4 mice) versus matched controls (4 mice). Based on these initial studies, three additional experiments each with 2 groups of mice each (control and $C3^{-/-}$ mice, $n=3-4$ /group) were conducted to evaluate 3 different Ad5Luc1 doses (2.3×10^9 , 4.0×10^9 , and 1.3×10^{10} v.p.). At various times after administration of Ad5Luc1, the mice were imaged using a bioluminescence imaging system (Xenogen, Inc.) to detect luciferase expression. Images were collected on mice oriented in the same position and always 10 min after intraperitoneal injection of 2.5 mg luciferin. The mice were maintained under enflurane anesthesia at 37 °C, with their ventral surfaces facing the CCD camera that was part of the imaging system. Imaging was performed several times on each mouse, beginning at 6 hr after Ad5Luc1 injection and continuing to day 34. Data acquisition times for imaging ranged from 20 sec to 10 min.

Figure 2: Liver light emission (luciferase expression) over time in the 3 experiments. Mice were intravenously dosed with Ad5Luc1 at (A) 2.3×10^9 v.p./mouse, (B) 4.0×10^9 v.p./mouse, and (C) 1.3×10^{10} v.p./mouse. The numbers adjacent to the wildtype data points indicate the fold greater expression for the wildtype group relative to the $C3^{-/-}$ group for that time point, with the “**” indicating statistical significance at $p < 0.05$. Each line is representative of 4 mice, except there were only 3 control mice in (B). Male mice (A) and female mice (B-C) were used. Light emission from the liver region (relative photons/sec) was measured using software provided by Xenogen, and the intensity represents the liver luciferase activity. This relationship was validated by comparing luciferase measurements from the live animals with independent measurements obtained from tissue homogenates as described.³⁰ These comparisons

were accomplished at termination by removal of liver and spleen (mice injected with 2.3×10^9 v.p.), followed by independent *in vitro* luciferase analyses as described. The validation also confirmed that the liver was responsible for >99% of the light emission that was detected in the liver region of the live mice using the Xenogen system.

Figure 3: Liver light emission (luciferase expression) over time in mice intravenously dosed with Ad5FFLuc1 (4.0×10^9 v.p./mouse). The numbers adjacent to the wildtype data points indicate the fold greater expression for the wildtype group relative to the C3^{-/-} group for that time point, with the “**” indicating statistical significance at $p < 0.05$. Each line is representative of 5 male mice.

Figure 4: Ad5Luc1-induced luciferase expression in TIB76 cells following treatment with various mouse sera. Ad5Luc1 aliquots (4.0×10^8 v.p., 0.02 ml) were incubated for 5 min (37° C) with 0.1 mL of fresh sera (wildtype, C1q^{-/-}, Factor B^{-/-}, or C3^{-/-}), PBS, or a mixture of wildtype and C3^{-/-} sera. Each mixture was diluted (3 mL) and 0.3 mL was incubated for 1 hr with adherent TIB76 cells in 24-well plates. Luciferase and protein assays were conducted after 22 hrs. Different letters indicate statistically significant differences in treatments at $p < 0.05$. These data are from one experiment, but are representative of data from experiments repeated on 3 different days.

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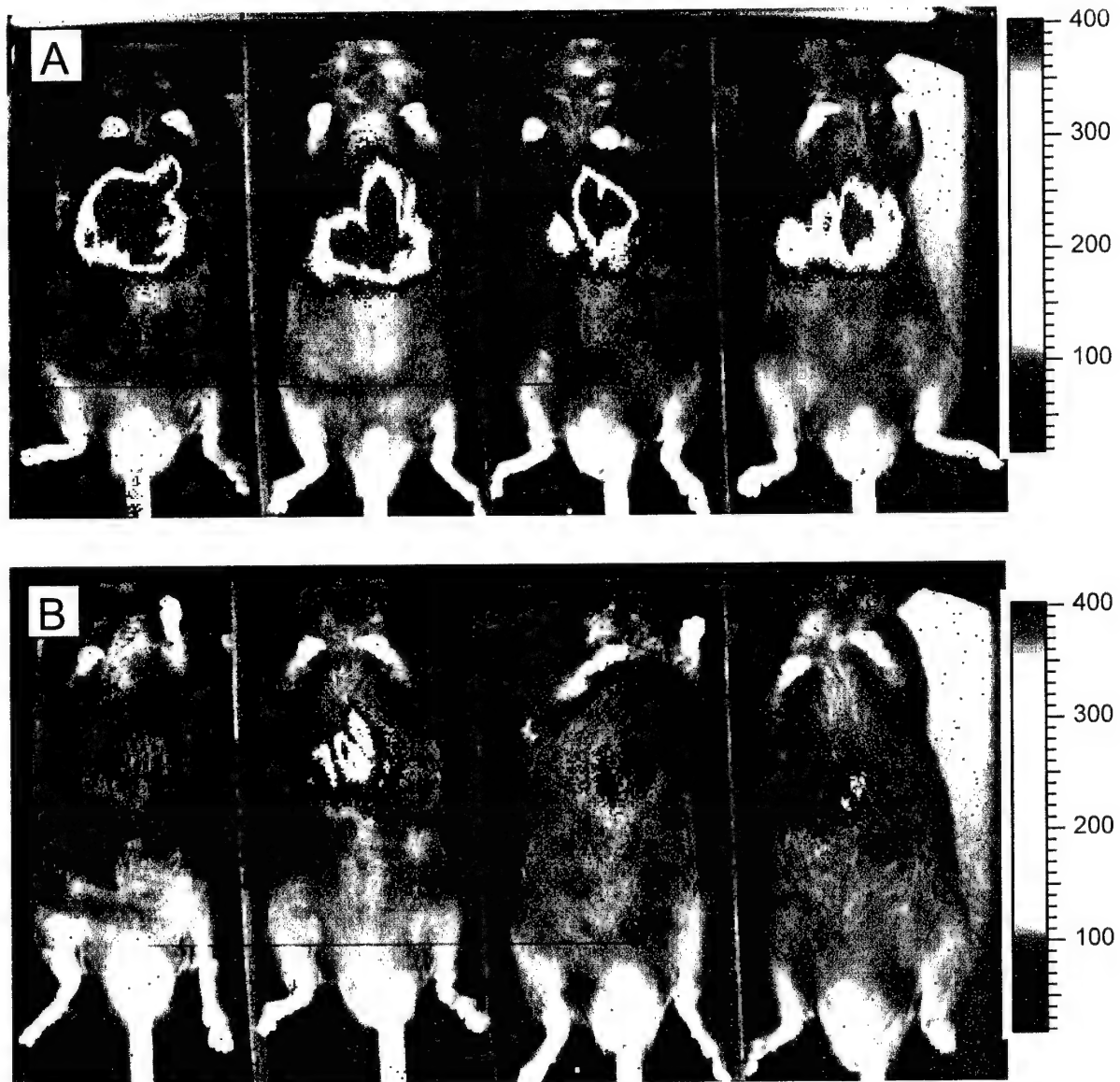


Figure 1

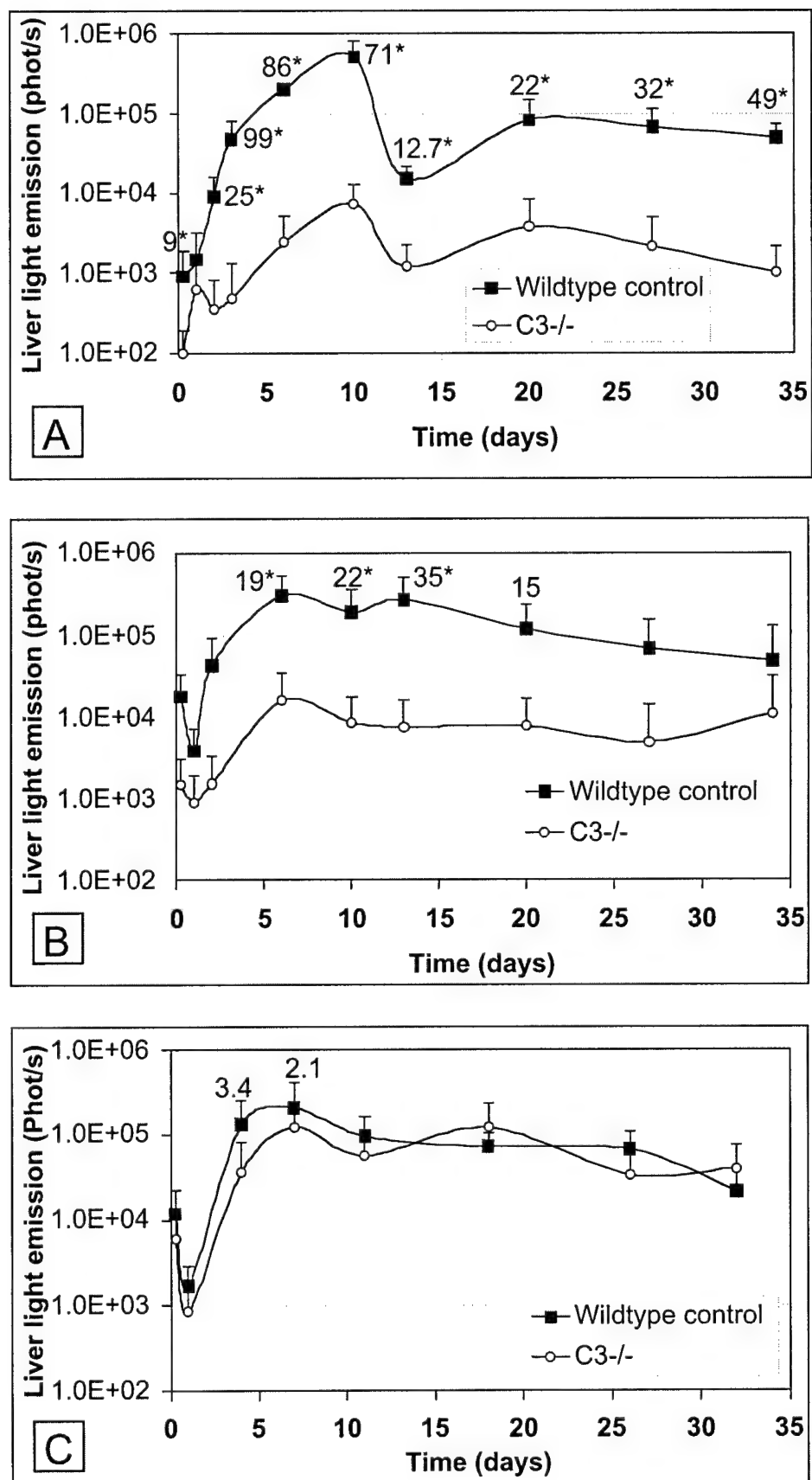


Figure 2

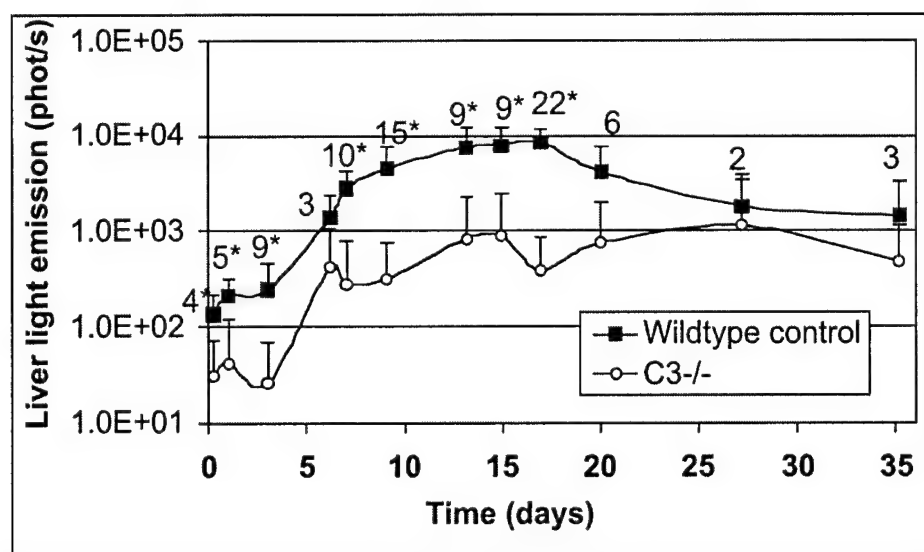


Figure 3

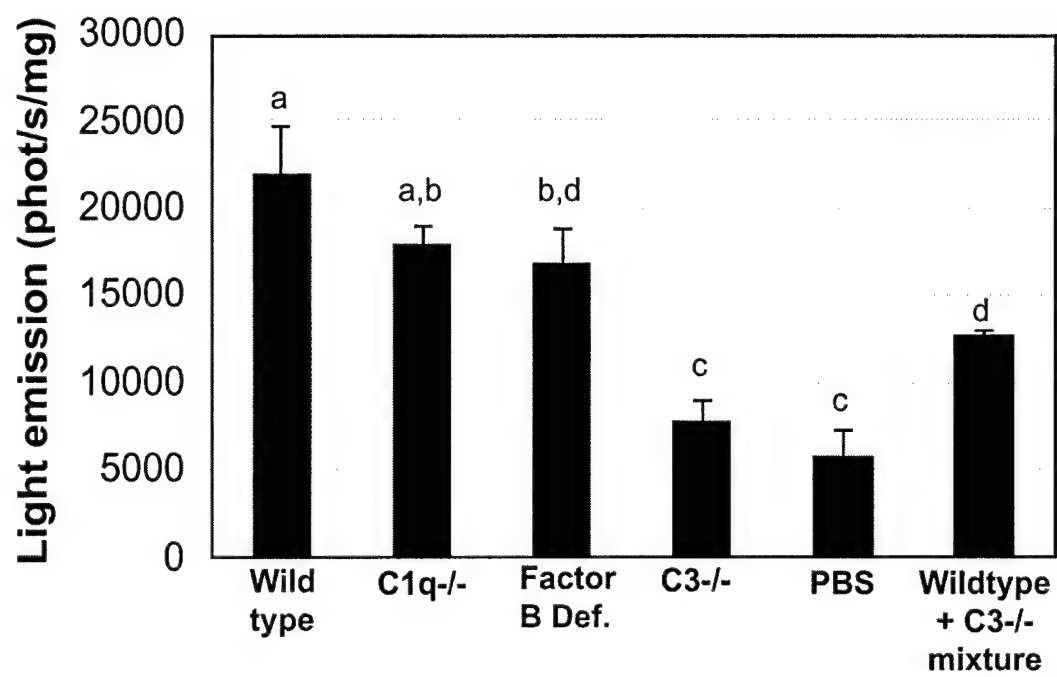


Figure 4

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Appendix 4
Chaudhuri, Tandra R., Ph.D.
DAMD17-02-1-0266

CURRICULUM VITAE

Date: April 28, 2004

Tandra R. Chaudhuri

Citizenship: USA

Foreign Languages: Bengali, Sanskrit

RANK/TITLE: Associate Professor, Department of Radiology
Co-Director, Multi-Modality Imaging Center

BUSINESS ADDRESS: Department of Radiology
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PROFESSIONAL CONSULTANTSHIPS:

Southeast Texas Radiation Oncology, Port Arthur, TX

EDUCATION:

George Washington University	MA	1970
University of Oklahoma	MS	1976
University of Calcutta	PhD	1983

LICENSURE: N/A

ACADEMIC APPOINTMENTS: (In reverse chronological order):

2000-present	Associate Professor Department of Radiology Co-Director, Laboratory for Multi-modality Imaging Assessment University of Alabama at Birmingham Birmingham, AL
1997-2000	Faculty Associate (UAB), Radiology - Molecular Imaging Development Lab
1995-2000	Director of Research/Consultant, Southeast Texas Radiation Oncology; Collaborator, Cancer Center of Port Arthur; Collaborator, University of Texas Health Science Center at San Antonio, Department of Nuclear Medicine; Collaborator (UAB), Radiology
1990-1995	Research Scientist (Associate Professor), University of Missouri

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1985-1990	Research Assistant Professor, University of Missouri
1984-1985	Research Associate, Michael Reese Hospital and Medical Center, University of Chicago
1981-1984	Research Associate, University of Oklahoma
1979-1981	Post-Graduate Research Fellow, University of Calcutta
1976-1978	Research Associate, University of Texas Health Science Center at San Antonio

AWARDS/HONORS:

Graduate Scholarship	University of Oklahoma 1974-1976
Research Fellowship	University of Calcutta 1979-1981

PROFESSIONAL SOCIETIES:

American Society of Gene Therapy
Society of Nuclear Medicine
American Society for Microbiology
American Association of Tropical Medicine and Hygiene
American Association for the Advancement of Science
Sigma Xi Society

UNIVERSITY ACTIVITIES:

University of Missouri:

1. Established Cell Culture and Immunochemistry Laboratory Facilities for University Researchers.
2. Survey of P-32 and other radioisotopes for University Researchers.
3. Mentor, Summers 1992-94, Undergraduate summer interns in NSF funded program.

UAB:

1. Co-established the Molecular Imaging Laboratory and Multi-Modality Imaging Assessment in the Department of Radiology, 1995-Present.
2. Established the cell culture laboratory in the Department of Radiology.

MANUSCRIPT REVIEWER:

Journal of Immunology
Journal of Bacteriology

MAJOR RESEARCH INTERESTS:

1. Early detection and treatment of prostate, ovarian, breast, lung, pancreatic and other cancers by Multi-Modality Imaging.
2. Efficacy of novel anti-cancer/anti-inflammatory drugs by imaging with radiotracer molecules.
3. Molecular mechanism of rheumatoid arthritis.
4. Non-invasive screening and imaging for the early detection of prostate, ovarian, breast, cervical and other cancers.

GRANT SUPPORT:

1. Neutron Activation of Sm²O₃ Tablets: Analyses and Clinical Studies, Investigators: PI: K Zinn, **TR Chaudhuri**, and S Morris; Procter and Gamble, \$8,500; April 1993 - December 1994.
2. Production and Purification of High Specific Activity Cu-64 for Clinical Studies, Investigators: PI: K. Zinn, **TR Chaudhuri**, and S Morris; Washington University, \$35,000; March 1993 - April 1995.
3. Nuclear Imaging of Adhesion Molecules in Inflammatory Disease. PI: KR Zinn, Co-Investigator: **TR Chaudhuri**. Agency: SNM Educ. & Research Foundation Type: Research grant. Period: 08/01/96 - 07/31/98, \$50,000. Goal: To develop radiolabeled peptides for imaging the *in vivo* expression of E-Selectin.
4. Selective Transduction for Early Detection and Therapy of Cancer. PI: DT Curiel. Consultant: **TR Chaudhuri**. Agency: NCI. Type: Contract. Period: 10/1/99 - 09/30/02, \$1,780,510. Goal: We propose the development of a novel system capable of selective transduction of target cells in the context of the clinical settings of: 1) post-treatment recurrent neoplastic disease and metastatic disease, and 2) early subclinical/undetected pre-neoplastic disease in susceptible cohorts.
5. Light based imaging for Breast Cancer. PI: **TR Chaudhuri**. Agency: UAB Cancer Center (Avon). Period: 9/1/2000 - 8/31/2002, \$250,000. Goal: Non-invasive detection and treatment of breast cancer.
6. *In vivo* Light-based Imaging for Early Detection and Monitoring of Ovarian Cancer. Ovarian Spore (Specialized Program of Research Excellence - Spore). PI: **TR Chaudhuri**. 01/01/02-01/01/03. \$50,000.
7. NCI, NIH. An *in vivo* Reporter System for Imaging Gene Transfer. PI: KR Zinn, Co-Investigator: **TR Chaudhuri**. Period: 7/6/1999 - 04/30/2004, \$155,000/yr. Goal: To develop a Tc-99m-based reporter system for *in vivo* imaging of gene transfer and to conduct therapy studies using Re-188-peptides, in combination with bicistronic vectors encoding cytosine deaminase.
8. NIH. Specialized Program of Research Excellence (SPORE) in Ovarian Cancer. PI: E Partridge, Co-Investigator: **TR Chaudhuri**. Agency: NIH. Type: Contract. Period: 9/30/99 - 09/29/04. Total Direct~\$100,000/year. Goal: **Dr. Zinn and Dr. Chaudhuri are directors on the *In vivo* Gamma-ray Imaging Core for Non-Invasive Imaging.**
9. Sankyo. Multi-modality Imaging Core, PPG, Overall PI, Koopman. PI: Zinn KR. Co-PI: **Chaudhuri TR**. Total Costs: \$150,000 DC/year Total Period 10/01/01 - 09/30/04.

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10. NCI, Infectivity Enhanced adenoviral vectors for ovarian cancer, total direct =\$1,232,741. PI: Ronald D. Alvarez; Co-Investigator, **Chaudhuri TR** . Total Period 8/9/2001 - 8/8/2004.
11. NIH, Enhanced CRAd for esophageal adenocarcinoma, PI: Masato Yamamoto, Co-Investigator: (5%) **TR Chaudhuri**. Total Direct=\$250,000/yr, total period 07/30/03-06/30/08.
12. HSF, Enhancement of the laboratory for multimodality imaging assessment. PI: Kurt R. Zinn, Co-Investigator: **TR Chaudhuri** Total=\$450,000, Total Period 03/01/03 to 02/28/05. **(NEW)**.
13. NIH, Career Development Award. Brain SPORE in molecular therapeutics for anaplastic glioma. PI: **TR Chaudhuri**. Total 65,250/year. Total Period: 06/01/03 to 05/31/05. Renewal for second year upon progress.
14. NIH. Breast SPORE. Co-Investigator: **TR Chaudhuri**. Total 50,000. Total Period: 10/01/03 to 09/30/04. Renewal for second year upon progress.
15. NIH grant. Comprehensive Cancer Center Core Support Grant. Instrument grants for small animal imaging equipment (Administrative Supplement). PI: AF LoBuglio. Co-Investigators: Zinn KR, **Chaudhuri TR**, Robbin ML. Total Costs: \$170,000. Total Period: 9/1/03 to 3/31/04. **(NEW)**.
16. Cystic Fibrosis Foundation. Cystic Fibrosis Gene Therapy Core Center, PPG, Overall PI, Sorscher: Development of Multimodality Approaches for Non-invasive Imaging of Rodent Lung. PI: **Chaudhuri TR**. Total Period: 05/01/03 - 04/30/04. Total Costs ~\$50,000. **(NEW)**.
17. NIH, SPORE in Pancreatic Cancer, PI: Vickers S, Co-Investigator: **TR Chaudhuri**, Imaging component of the animal models core. Period: 06/01/03 to 08/31/08, Total Costs: 650,000 direct/year (\$40,000/year for imaging). **(NEW)**. Renewal for second year upon progress.
18. NIH, Brain SPORE Developmental Project. PI: Wu, H. **Director/Mentor: Chaudhuri, TR**. Transcytosis-targeted adenoviral vector for brain cancer. Period: 01/01/2004 to 12/31/2004, Total Costs: \$ 45,000. **(NEW)**. Renewal for second year upon progress.
19. NIH, Targeted Adenovirus Vectors for Gene Therapy of prostate cancer. PI: Victor Krasnykh, Co-Investigators, Kurt R. Zinn and **Tandra R. Chaudhuri**. Period: 07/01/03 to 06/30/08, \$1,809,800 total costs. (funded and moved from UAB). **(NEW)**

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20. DOD grant. Non-invasive Dual Modality Imaging for the Early Detection and Monitoring of Breast Cancer During Therapy. PI: **TR Chaudhuri**. Total Direct=\$100,000/yr, 01/01/02 – 12/31/04.
21. NIH. Ovarian SPORE Developmental Project. Early detection of ovarian cancer by high frequency ultrasonography. PI: Zinn KR, Co-Investigator: **Chaudhuri, TR**. Total Costs: 50,000. Total Period: 01/30/04 to 01/29/05. Renewal for second year upon progress.
22. NIH. Ovarian SPORE Developmental Project. Exploring Human Gamma Delta T-cells for the Immunotherapy of Ovarian Cancer. PI: Lopez, Richard. Co-Investigator: **Chaudhuri, TR. (NEW)**. Total Costs: 50,000. Total Period: 01/30/04 to 01/29/05. Renewal for second year upon progress.

GRANTS PENDING:

1. NIH, RO1 Competitive Continuation, Specialized Program of Research Excellence (SPORE) in Ovarian Cancer. PI: Edward Partridge; **TR Chaudhuri is Co-Investigator and Co-Director of Imaging Core (10% effort)**, Total Direct ~ \$7,000,000, 05/01/04-04/30/09. **(NEW)**.
2. NIH. Southern Molecular Imaging Link (SMIL). PI: Zinn KR. Co-Investigator: **Chaudhuri TR**. Total Period: 7/1/04 - 6/30/09. Total Costs: \$899,730/5 years. **(NEW)**.

GRANTS IN PREPARATION:

1. Multi-Modality Imaging to Study the Molecular Mechanism of Bone Metastasis in Breast and Prostate Cancers. PI: **TR Chaudhuri**.

Research Support for Students at the University of Missouri:

1. Scientific Applications of Neutrons at a University Research Reactor, Investigators: F. Ross, S. Morris, K. Zinn, M. Glascock, J. Farmer, R. Berliner, G. G. Ehrhardt, A. Ketrang, B. Yelon, **T. Chaudhuri**, H. Neff, and H. Kaiser, NSF; \$165,000, 1992-1995.

Other: Invention Disclosures:

1. Disclosed (UAB): The human type 2 somatostatin gene in combination with a radiolabeled somatostatin-avid peptide functions as an *in vivo* reporter system for imaging gene transfer. Inventors: K Zinn, D Buchsbaum, B Rogers, **TR Chaudhuri**, March 1999.
2. Disclosed (UAB): An *in vivo* reporter system for imaging gene transfer, Inventor: K. Zinn, **T.R. Chaudhuri**. March 1999.

3. Disclosed (UAB): Novel Promoter/Reporter Screening System. K Zinn, **TR Chaudhuri**, B Rogers, October 1999.
4. Method for production of luciferase-positive cancer cell lines for imaging, K. Zinn, **T.R. Chaudhuri**, and S. Ponnazhagan. 1-21-03.
5. In vivo inflammation monitor. K. Zinn, **T.R. Chaudhuri**, M. Yamamoto. 2-7-03.
6. Disclosed (UAB): Application for Fluorescent Stereomicroscopic Imaging for the early detection and treatment monitoring in non-invasive way. **Chaudhuri, TR** , Zinn. KR.

Other: PATENTS granted and submitted

1. U.S. Provisional Application: Methods and Compositions for *in vivo* inflammation monitoring. Inventor: K. Zinn, **T. Chaudhuri**.
2. U.S. Submitted: Gene Transfer Imaging and Uses Thereof, Continuation, Inventors: D. Buchsbaum, K. Zinn, B. Rogers, **T. Chaudhuri**, D. Curiel.

MANUSCRIPTS PUBLISHED:

1. **Chaudhuri TR** and Lancaster JH. Cell surface components involved in E. coli conjugation. Pili. 235-239, 1978.
2. Johanson W, **Chaudhuri TR** and Woods DE. Association of respiratory tract colonization with gram negative bacilli to epithelial cell. Journal of Infectious Diseases 139:667-673, 1979.
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95. **T. R. Chaudhuri**, V. N. Krasnykh, Z. Cao, A. Stargel, P. L. Simhadri, N. Belousova, K. R. Zinn. Combined fluorescent and bioluminescent imaging for the detection of human prostate cancer metastasis in nude mice after intravenous administration of replication competent Ad vector. Journal of International Conference on Gene Therapy of Cancer, December 10-14, 2003, San Diego, California.
96. **T. R. Chaudhuri**, V. N. Krasnykh, Z. Cao, A. Stargel, P. L. Simhadri, N. Belousova, K. R. Zinn. Intravenous administration of adenoviral vector detected metastatic breast cancer by bioluminescent imaging in live mice. Journal of International Conference on Gene Therapy of Cancer, December 10-14, 2003, San Diego, California.
97. **Chaudhuri TR**, Viral delivery of light-based reporters for detection and monitoring of disease. Mol Imaging Biol 6(2): 69, 2004.
98. Dugger KJ, Zinn KR, **Chaudhuri TR**, Stargel A, Reason G, Weaver CT. Bioluminescence imaging of activated T cells following adoptive transfer. Mol Imaging Biol 6(2): 78, 2004.
99. Zinn KR, **Chaudhuri TR**, Oh P, Stargel A, Schnitzer JE. High resolution single photon emission computed tomography/ computed tomography evaluation of an antibody targeted to lung endothelium. Mol Imaging Biol 6(2): 84, 2004.

ABSTRACTS SUBMITTED:

1. **Chaudhuri TR**, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum and Kurt R. Zinn. Bioluminescence imaging of non-palpable breast cancer xenografts during treatment with TRA-8, an anti-DR5 antibody and chemotherapy. AACR. March, 2004, in Orlando Florida. **(Accepted)**
2. Juan L. Contreras, **Chaudhuri, Tandra R.** Amanda Stargel, Hongju Wu, Cheryl A. Smith, Guadalupe Bilbao, David T. Curiel, Devin Eckhoff, Kurt R. Zinn. Invivo

Quantitative Noninvasive Bioluminescence Imaging of Intraportal Transplanted Islets. American Transplant Congress.

3. **Chaudhuri TR**, Zhihong Cao, Selvarangan Ponnazhagan, Amanda Stargel, Pushpa L. Simhadri, Tong Zhou, Albert F. LoBuglio, Donald J. Buchsbaum, and Kurt R. Zinn. Detection of disseminated breast cancer growth and treatment response using non-invasive bioluminescence imaging (BI). ASCO, June 2004, in New Orleans, Louisiana.

SCIENTIFIC PAPERS PRESENTED AT NATIONAL AND INTERNATIONAL MEETINGS:

1. **Chaudhuri TR** and Lancaster JH. A possible mechanism of Escherichia coli K12 conjugation. National meeting of American Society for Microbiology, New Orleans, 1977.
2. **Chaudhuri TR**, Lancaster JH, and Lou R. Diagnosis of Hepatitis by Tan protein. Interscience conference on anti-microbial agents and chemotherapy, Chicago, Illinois, 1978.
3. **Chaudhuri TR** and Lancaster. Role of F-pili in bacterial conjugation; National meeting of American society for Microbiology, Las Vegas, 1978.
4. Lancaster JH and **Chaudhuri TR**. Cell surface components involved in Escherichia coli conjugation. Pili. International conference on pili. Halifax, Canada, 1978.
5. **Chaudhuri TR**. Protein-LPS interaction in bacterial conjugation. International congress of cell biology, Berlin, West Germany, 1980.
6. **Chaudhuri TR**, Talukder G, Sharma, A, et al. Interaction between protein and LPS in the mechanism of conjugation in gram negative bacteria. National Congress of Science, Calcutta, India, 1983.
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8. **Chaudhuri TR**, Green T. A sensitive urea-silver stain method for detecting trace quantities of proteins in polyacrylamide gels. University of Missouri, Columbia, Molecular Biology Meeting, 1986.
9. **Chaudhuri TR**, Green T. Characterization of merozoite surface antigen (pf 43 mz) isolated from Plasmodium falciparum. University of Missouri, Columbia, Molecular Biology Meeting, 1986.

10. **Chaudhuri TR** and Green T. Immunochemical analysis of merozoite surface antigen (Pf43 mz) isolated from *Plasmodium falciparum*. American Society for Microbiology National Meeting, Atlanta, Georgia, 1987.
11. **Chaudhuri TR**, Clark WA, Schroff S. Effects of thyroid hormone and adriamycin on the growth of cultured heart cells. FASEB, St. Louis, Mo. 1987.
12. **Chaudhuri TR** and Green T. Synthetic polypeptides produce specific antibodies to merozoite of *plasmodium falciparum*. American Society of Tropical Medicine and Hygiene, Los Angeles, California, 1987.
13. Zinn KR, **Chaudhuri TR**, et al. Identification and comparisons of selenoproteins in rat brain and mouse neuroblastoma cells. FASEB, Atlanta, GA, April 1991.
14. Zinn KR, **Chaudhuri TR**, et al. Comparisons of Se-75-labeled proteins from Neuroblastoma (N2A), Hepatoma (H4IIE), and Fibroblasts (3T6) grown in low selenium media. Presented at the Fifth International Symposium on Selenium in Biology and Medicine, Nashville, TN, July, 1992.
15. **Chaudhuri TR**, Zinn KR, Morris JS, et al. Detection of ovarian cancer by Au-198-labeled human monoclonal antibody. Fourth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer, Princeton, NJ, Sept. 1992.
16. **Chaudhuri TR**, Zinn KR, Morris JS, et al. Characterization of human monoclonal antibody against ovarian cancer. Fourth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer, Princeton, NJ, Sept., 1992.
17. **Chaudhuri TR**. Radioimmunodiagnosis and treatment of human ovarian cancer. Invited speaker - Regional conference of American Society for Microbiology and Immunology, Columbia, MO, April 1992.
18. Zinn KR, **Chaudhuri TR**, Cheng TP, et al. Production of No-Carrier-added Cu-64 from Zinc metal irradiated under Boron shielding. Fourth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer, Princeton, NJ, Sept. 1992.
19. **Chaudhuri TR**, Zinn KR, Morris JS, et al. Radioimmunodetection of ovarian cancer by Au-198-labeled human monoclonal antibody against ovarian cancer. Missouri Valley and Central Chapter meeting, Society of Nuclear Medicine, St. Louis, MO, October, 1992.
20. **Chaudhuri TR**, Zinn KR, Morris JS. Radio-immuno-detection of ovarian cancer by Au-198-labeled human monoclonal antibody *in vitro*. International Conference on

Monoclonal Antibody Immunoconjugates for Cancer, San Diego, California, March, 1993.

21. **Chaudhuri TR**, Zinn KR, Morris JS, et al. Characterization of human monoclonal antibody against ovarian cancer. International Conference on Monoclonal Antibody Immunoconjugates for Cancer, San Diego, California. March, 1993.
22. Zinn KR, **Chaudhuri TR**, Morris JS, Cheng TP and Meyer W. Production and purification of high specific activity Cu-64 for PET imaging. Society of Nuclear Medicine Annual Meeting, Toronto, Canada, June, 1993.
23. Zinn KR, **Chaudhuri TR**, Morris JS, et al. Specific activity Cu-64 for PET imaging. Society of Nuclear Medicine Annual Meeting, Toronto, Canada. June, 1993.
24. **Chaudhuri TR**, Zinn KR, Morris JS, et al. Analyses of human monoclonal antibody against ovarian cancer antigen. Fourth Biennial Meeting of the International Gynecologic Cancer Society, Stockholm, Sweden. August-September, 1993.
25. **Chaudhuri TR**, Zinn KR, Morris JS, et al. *In vitro* detection of ovarian cancer using Au-198-labeled human monoclonal antibody. Fourth Biennial Meeting of the International Gynecologic Cancer Society, Stockholm, Sweden. August-September, 1993.
26. **Chaudhuri TR**, Zinn KR, Morris JS, McDonald GA, Llorens AS and Chaudhuri TK. Cytotoxicity test of antibodies against human ovarian cancer cell surface antigen. Ninth International conference on Monoclonal Antibody Immunoconjugates for Cancer, San Diego, CA, March 1994.
27. Zinn KR, **Chaudhuri TR**, Morris JS, Cheng TP. Copper-64: Production and medical applications. 208th ACS National Meeting, Aug. 21-25, 1994.
28. Zinn KR, **Chaudhuri TR**, Morris JS, Cheng TP and Meyer WA, Jr. Experience with production of Cu-64: A novel radionuclide for PET imaging and potential agent for radioimmunotherapy. Ninth International conference on Monoclonal antibody Immunoconjugates for Cancer, San Diego, CA, March, 1994.
29. **Chaudhuri TR**, Zinn KR, Morris JS, McDonald GA, Llorens AS and Chaudhuri TK. Development and characterization of radiolabeled human monoclonal antibody against ovarian cancer. Annual meeting of Society of Nuclear Medicine, Orlando, FL, June, 1994.
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on Monoclonal Antibody Immunoconjugates for Cancer, San Diego, CA, March, 1995.

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32. **Chaudhuri TR**, Zinn KR, Llorens and Chaudhuri TK. Human monoclonal antibody against Ovarian Cancer. FASEB, 1995.
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34. Zinn KR, **Chaudhuri TR**, Mountz JD, Zhou T, Mountz JM. Evaluation of E-Selectin binding-peptides for imaging inflammatory disease. National Scientific Meeting of Society of Nuclear Medicine. Denver, CO, June, 1996.
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47. Zinn KR, Buchsbaum DJ, **Chaudhuri TR**, Mountz JM, Curiel DT, Krasnykh VN, Rogers BE. Simultaneous *In vivo* Imaging of Thymidine Kinase and Somatostatin Receptor Expression after Gene Transfer with an Adenoviral Vector Encoding Both Genes. 2000.
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49. Rogers BE, Buchsbaum DJ, **Chaudhuri TR**, Krasnykh VN, Curiel DT, Zinn KR. *In Vitro* and *In Vivo* Imaging of Thymidine Kinase and Somatostatin Receptor Subtype 2 Expression Following Gene Transfer with an Adenoviral Vector. Annual Meeting of Society of Nuclear Medicine. 2000.

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53. **Chaudhuri TR**, Mountz JM, Rogers BE, Partridge EE, Zinn KR. Early Detection of ovarian cancer by light-based imaging. Society of Gynecologic Oncology Annual Meeting, Nashville, TN, March, 2001.
54. **Chaudhuri TR**, Mountz JM, Rogers BE, Robinson GD, Edward E. Non-invasive Light-based Imaging of GFP-Positive Ovarian Xenografts. American Society of Gene Therapy Annual Meeting, Seattle, WA, April, 2001.
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56. **Chaudhuri TR**, Krasnykh VN, Belousova N, Zinn KR, Buchsbaum DJ, Mountz J, Curiel DT, Rogers BE. An Ad-Based Strategy for Imaging, Radiotherapy, and Enhanced Tumor Killing. American Society of Gene Therapy, Annual Meeting. Seattle, WA, April, 2001.
57. Zinn KR, **Chaudhuri TR**, Belousova N, Buchsbaum DJ, Mountz JM, Curiel DT, Krasnykh MN, Rogers BE. Dose-response comparison for two *in vivo* reporter genes for imaging transfer. American Society of Gene Therapy. Annual Meeting, Seattle, WA, April, 2001.
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60. **Chaudhuri TR**, Cao Zhihong, Rogers BE, Partridge EE and Zinn KR. Light-based Imaging of ovarian cancer. 9th Workshop of SPORE, Wash. D.C., July 15-17, 2001.
61. Zinn KR and **Chaudhuri TR**. *In vivo* Imaging of Ovarian Cancer. Invited 9th Workshop of SPORE, Wash. D.C., July 15-17, 2001.
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64. Zinn KR, **Chaudhuri TR**, Belousova N, Davis AJ, Mountz, Jr., JD, Mountz JM, Curiel DT, Krasnykh VN. *In Vitro* and *In vivo* Imaging of 99mTc-Labeled Recombinant Adenovirus. American Society of Gene Therapy, Annual Meeting. Seattle, WA, April, 2001.
65. **Chaudhuri TR**, Rogers BE, Mountz JM, Partridge EE, Zinn KR. Non-invasive imaging of ad-vector-transfected ovarian xenografts. European Association of Nuclear Medicine, 2001.
66. **Chaudhuri TR**, Mountz JM , Rogers, BE and Zinn KR. *In vivo* Light-based Imaging of Ad-vector-transfected Ovarian Xenografts. International Congress of European Society of Nuclear Medicine, Naples, Italy, August, 2001.
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68. **Chaudhuri TR**, Mountz JM, Rogers BE, Robinson GD; LoBuglio AF and Zinn KR. *In Vivo* Imaging for the Early Detection and Monitoring of Human breast Cancer in Animal Model During Therapy. International Congress of European Society of Nuclear Medicine, Naples, Italy, August, 2001.
69. Zinn KR, **Chaudhuri TR**, Belousova N, Davis AJ, Mountz Jr JD, Mountz JM, Curiel DT, Krasnykh VN. Imaging Evaluation of Tc-99m-Labeled Recombinant Adenovirus. International Congress of European Society of Nuclear Medicine, Naples, Italy, 2001.

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72. **Chaudhuri TR**, Cao Z, Rodriguez-Burford C, LoBuglio AF, Zinn KR. *In vivo* Optical Imaging of Angiogenesis in Breast Cancer Xenografts. American Society of Gene Therapy, Annual Meeting. Seattle, WA, April, 2002.
73. **Chaudhuri TR**, Krasnykh VN, Cao Z, Zinn KR. Dualistic Genetic Reporter System for Early Diagnosis and Monitoring of Ovarian Cancer American Society of Gene Therapy, Annual Meeting. Seattle, WA, April, 2002.
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75. Zinn KR, Cao Z, Ma Z, **Chaudhuri TR**. Detection of Ovarian Cancer with a New Plasmid Vector Encoding Two Reporters: Blood-Based Screening Combined with Imaging. American Society of Gene Therapy, Annual Meeting. Seattle, WA, April, 2002.
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77. Hemminki A, Zinn KR, Bauerschmitz GJ, **Chaudhuri TR**, Barnes, MN, Alvarez RD, Curiel DT. Integrin targeted adenoviruses for ovarian cancer gene therapy. Proceedings of ASCO, 2002.
78. Buchsbaum DJ, Zinn KR, Alvarez RD, **Chaudhuri TR**, Axworthy DB, Schultz J, Theodore LJ, Carpenter M, Khazali M, Meredith RF, Partridge EE, LoBuglio AF. Validating the utility of a novel pretargeting approach for intraperitoneal (IP) radioimmunotherapy (RIT). American Society of Clinical Oncology, Orlando, FI, 2002.
79. **Chaudhuri TR**, Cao, Z, Davis AJ, Della Manna D, Rodriguez-Burford C, Robinson GD, Partridge EE, Zinn KR. Treatment monitoring of microscopic ovarian cancer in live mice. American Society of Clinical Oncology, Orlando, FI, 2002.

80. Zinn KR, Cao Z, Partridge EE, **Chaudhuri TR**. Noninvasive detection of ovarian cancer by simultaneous light-based and gamma-camera imaging. American Society of Clinical Oncology, Orlando, FL, 2002.
81. Zinn KR, **Chaudhuri TR**, Krasnykh VN, Curiel DT, Reynolds PN. *In vivo* imaging of Tc-99m-labeled ad vector redirected to lung following intravenous dosing. Eur J Nucl Med Mol Imaging. (Presented at 2002 meeting of the European Association of Nuclear Medicine, Vienna, Austria, 09/02/02).
82. **Chaudhuri TR**, Cao Z, Partridge EE, Zinn KR. Dual modality imaging for treatment monitoring of microscopic ovarian cancer in live mice. Eur J Nucl Med Mol Imaging. (Presented at 2002 meeting off the European Association of Nuclear Medicine, Vienna, Austria, 09/04/02).
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84. Zinn KR, Cao Z, Rodriguez-Burford C, **Chaudhuri TR**. Validation of non-invasive light-based imaging by gamma camera imaging in prostate cancer xenografts. Eur J Nucl Med Mol Imaging (Presented at 2002 meeting of the European Association of Nuclear Medicine, Vienna, Austria, 09/04/02).
85. **Chaudhuri TR**, Cao Z, Ma Z, Zinn KR. Optical imaging combined with blood-based screening by a new plasmid vector for the detection of ovarian cancer. Eur J Nucl Med Mol Imaging (Presented at 2002 meeting of the European Association of Nuclear Medicine, Vienna, Austria, 09/04/02).
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88. **Chaudhuri TR**, Cao Z, LoBuglio AF, Zinn KR. *In vivo* imaging of angiogenesis in breast cancer xenografts during therapy. Breast Cancer Res Treat. (presented at the 25th Annual San Antonio Breast Cancer Symposium, December 2002).

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90. **Chaudhuri TR**, Cao Z, Krasynkh VN, Stargel AV, Belousova N, Zinn KR. Metastatic breast cancer is detected by bioluminescence imaging in live mice using an intravenous Ad vector. Mol Ther. (presented at the 6th Annual ASGT meeting, June 2003).
91. **Chaudhuri TR**, Krasynkh VN, Cao Z, Stargel AV, Belousova N, Zinn KR. Detection of human prostate cancer metastasis in nude mice after intravenous administration of Ad vector: combined fluorescence and bioluminescence imaging. Mol Ther. (presented at the 6th Annual ASGT meeting, June 2003).
92. Zinn KR, **Chaudhuri TR**, Stargel AV, Kumar S, Ponnazhagan S. Gamma camera imaging of Tc-99m-labeled AAV vector followed by bioluminescence imaging of luciferase transgene expression. Mol Ther. (presented at the 6th Annual ASGT meeting, June 2003).
93. Anna Kanerva, Kurt R. Zinn, **Tandra R. Chaudhuri**, Kah-Whye Peng, David T. Curiel, Akseli Hemminki. Enhanced Therapeutic Efficacy for Ovarian Cancer with a Serotype 3 Receptor Targeted Oncolytic Adenovirus. Mol Ther. (presented at the 6th Annual ASGT meeting, June 2003).
94. Zinn K, FW van Ginkel, EJ Sorscher, A Stargel, **TR Chaudhuri**. Non-invasive imaging of genetic reporters in rodent lung following adenoviral gene delivery. *Pediatric Pulmonary* (presented at the annual CF meeting in Anaheim CA, Oct 15-18, 2003).
95. **T. R. Chaudhuri**, V. N. Krasnykh, Z. Cao, A. Stargel, P. L. Simhadri, N. Belousova, K. R. Zinn. Combined fluorescent and bioluminescent imaging for the detection of human prostate cancer metastasis in nude mice after intravenous administration of replication competent Ad vector. International Conference on Gene Therapy of Cancer, Dec-10-14, 2003, San Diego, California
96. **T. R. Chaudhuri**, V. N. Krasnykh, Z. Cao, A. Stargel, P. L. Simhadri, N. Belousova, K. R. Zinn. Intravenous administration of adenoviral vector detected metastatic breast cancer by bioluminescent imaging in live mice. International Conference on Gene Therapy of Cancer, Dec-10-14, 2003, San Diego, California.
97. **Chaudhuri TR**, Viral delivery of light-based reporters for detection and monitoring of disease. Annual Conference of the Academy of Molecular Imaging, March 27-31, 2004, Orlando, Florida.

98. Dugger KJ, Zinn KR, **Chaudhuri TR**, Stargel A, Reason G, Weaver CT. Bioluminescence imaging of activated T cells following adoptive transfer. Annual Conference of the Academy of Molecular Imaging, March 27-31, 2004, Orlando, Florida.
99. Zinn KR, **Chaudhuri TR**, Oh P, Stargel A, Schnitzer JE. High resolution single photon emission computed tomography/ computed tomography evaluation of an antibody targeted to lung endothelium. Annual Conference of the Academy of Molecular Imaging, March 27-31, 2004, Orlando, Florida.

SCIENTIFIC PAPERS PRESENTED AT NATIONAL AND INTERNATIONAL MEETINGS: (No published abstract)

1. **Chaudhuri TR**. Mechanism of gene transfer in gram negative bacteria. University of Calcutta; Science Congress, Calcutta, India, Nov. 1981.
2. **Chaudhuri TR**. Protein-Polysaccharide interaction in the process of gene transfer in gram negative bacteria. University of Texas in San Antonio, Texas; 1977.
3. Rogers BE, Buchsbaum DJ, **Chaudhuri TR**, Krasnykh VN, Curiel DT, Zinn KR. *In Vitro* and *In vivo* Imaging of Thymidine Kinase and Somatostatin Receptor Subtype 2 Expression Following Gene Transfer with an Adenoviral Vector.

SCIENTIFIC PRESENTATIONS AT LOCAL AND REGIONAL MEETINGS:

1. **Chaudhuri TR**, Zinn KR, Morris JS, McDonald GA, Llorens AS, and Chaudhuri TK. Radioimmuno-detection of Ovarian Cancer by Au-198-Labeled Human Monoclonal Antibody. (presented at the Missouri Valley and Central Chapter meeting, Society of Nuclear Medicine, Oct 16-18, 1992, St. Louis, MO).
2. **Chaudhuri TR**, Zinn KR, McDonald GA, and Chaudhuri TK. Analysis of Human Monoclonal Antibody Against Ovarian Cancer. (presented at the Missouri Valley and Central Chapter meeting, Society of Nuclear Medicine, Oct 16-18, 1992, St. Louis, MO).
3. Zhihong Cao, Kurt R. Zinn, Amanda Stargel, Pushpa L Simhadri, Tong Zhou, Selvarangan Ponnazhagan, Albert F. LoBuglio, Donald J. Buchsbaum and **Tandra R. Chaudhuri**. Non-invasive imaging of non-palpable breast cancer xenografts during therapy. 2003 Annual Research Retreat. (presentation at the Comprehensive Cancer Center. October 2003 UAB). **Awarded for outstanding work in cancer research.**
4. **Chaudhuri TR**. Multi-modality Imaging of Metastasized Breast Tumors in Mice Brain. 2004 Inter Brain SPORE conference, Jan 22-23, UAB.

5. **Chaudhuri TR.** Efficacy of TRA-8 in breast cancer xenografts by bioluminescent imaging. 2003 SANKYO's annual meeting. November, UAB.

INVITED LECTURES

1. Regional conference of American Society for Microbiology, University of Missouri, Columbia, MO, March, 1993. Title: Radioimmunodetection and radioimmunotherapy for ovarian and breast cancers. **Chaudhuri TR.**
2. International Isotope Society, 7th meeting of the U.S. Central Section, Oct. 7, 1994. Title: Review of Monoclonal Antibody Applications in Nuclear Medicine. **Chaudhuri TR.**
3. University of Calcutta, India. Oct. 1981. Title: Mechanism of gene transfer in gram negative bacteria. **Chaudhuri TR.**
4. Light-based and gamma camera imaging for the early detection and monitoring of ovarian cancer. July 2001, Wash. D.C. SPORE meeting. **Chaudhuri TR.**
5. **Chaudhuri, TR.** Invited Speaker. Academy of Molecular Imaging, March 2004, in Orlando, Florida.

TEACHING/SERVICE ACTIVITY: *In vivo* gamma-ray imaging core facility

ACADEMIC SERVICE:

1. Served as an Active member of Executive Committee of Ovarian SPORE for grant review.
2. Served as an Active member of Executive Committee of Brain SPORE for grant review.
3. Reviewed Breast SPORE grant.

TEACHING ACTIVITIES/FORMAL LECTURES (at UAB):

STUDENTS ADVISED AT THE UNIVERSITY OF MISSOURI: UNDER GRADUATES

1. Brent Bell, Summer 1991 REU program, Project: Differential Solubility of Se-75-Labeled Proteins, Mentors: K. Zinn and **T. Chaudhuri.**
2. Maggie Stammeyer, Summer 1992 REU program, Project: A Procedure for the Study of Trace Element Binding to Proteins using High Specific Activity Cu-64. Mentors: K. Zinn and **T. Chaudhuri.**
3. Jeffery Johnson, Summer 1993 REU program, Project: Genetic Variation in Copper-binding Proteins in Rat Brain, Mentors: K. Zinn and **T. Chaudhuri.**
4. Jennifer Johnson, Summer 1993 REU program, Project: Purification of Monoclonal Antibody, Mentors: K. Zinn and **T. Chaudhuri.**

GRADUATE - MS DEGREE

1. Karalyn Littlefield, MS, 1990-1992, Development of nutrition and food science. Project: *In vitro* studies of anti-cancer drugs.

GRADUATE - PhD DEGREE

1. Joseph Temenak, PhD Department of Molecular Microbiology and Immunology, University of Missouri-Columbia. Project: Molecular immunology in infectious disease.
2. Lori Pellet, PhD Nutrition and food science

Technical and Professional Staff Trained and Other Students Assisted at Univ. of Missouri and at UAB:

1. Madeline Mason, Research Reactor, University of Missouri. Trained in cell culture and immunology research.
2. Wu Qi, UAB, Division of Nuclear Medicine. Trained in cell culture and *in vitro* experiments.
3. Zhu Min. UAB, Division of Nuclear Medicine. Trained in cell culture and *in vitro* experiments.
4. Gloria Robinson, Research Assistant, Trained in cell culture and *in vitro* research. Department of Radiology, UAB.
5. Ashley Davis, Department of Radiology, UAB, Trained in Molecular Imaging Research. Department of Radiology, UAB.
6. Zhihong Cao, MD, Department of Medicine, UAB. Research Associate.
7. Cristina Rodriguez-Burford, PhD, Research Associate. Dept of Radiology, UAB.
8. Amanda Stargel, Research Assistant. Department of Medicine, UAB.
9. Xiang Feng, MD, Research Associate. Department of Radiology, UAB.
10. Pushpa Simhadri, MS. Clinical Data Analyst. Department of Medicine, UAB.
11. Hongju Wu, PhD. Research Assistant Professor, Department of Radiology, UAB.
12. Glorisa Reason, MS, Research Technician. Department of Medicine, UAB.